A Tumor-Associated Antigen in Carcinoma of the Pancreas Defined by Monoclonal Antibody B72.3

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A retrospective analysis of 25 primary adenocarcinomas of the pancreas, 16 metastatic pancreatic tumors, 8 cases of chronic pancreatitis, and 3 adult normal pancreas was performed to ascertain the reactivity of monoclonal antibody (MAb) B72.3 to malignant and nonneoplastic pancreatic lesions. Formalin-fixed, paraffin-embedded sections of pancreas were evaluated by immunohistochemical techniques (avidin–biotin–peroxidase complex [ABC] method). Twenty-one of 25 malignant primary tumors were reactive, and all 16 metastatic sites expressed the B72.3 antigen. In contrast, all cases of pancreatitis and normal pancreas were either weakly reactive or nonreactive. Ten malignant and two benign pancreatic fine-needle aspirates provided results similar to those seen with fixed tissues. Because MAb B72.3 has selective reactivity for primary and metastatic pancreatic cancer, it may be of value as a diagnostic adjunct in cytologic examination or for radioimmunodetection of regional and/or distant metastases of adenocarcinoma of the pancreas. (Key words: Monoclonal antibody; Immunohistochemistry; Pancreatic cancer) Am J Clin Pathol 1988;89:160–167

IN PREVIOUS immunohistochemical studies with formalin-fixed, paraffin-embedded tissues, monoclonal antibody (MAb) B72.3 has exhibited reactivity with a wide spectrum of carcinomas, including 94% of colonic adenocarcinomas, 84% of invasive ductal carcinomas of the breast, 96% of non–small cell lung carcinomas, and 100% of common epithelial ovarian carcinomas, but very limited reactivity with the respective normal tissues.[1] Its selectivity has been further supported by its failure to react with nonepithelial malignancies. The antigen to which MAb B72.3 reacts is a mucin-like glycoprotein (TAG-72) with a molecular weight of approximately 1×10^6 daltons.[11] It is expressed in several endodermally derived organs in the fetus but not in fetal pancreas.[21] In gastrointestinal malignancies, including pancreatic cancer, shed antigen is present in the serum.[18] Our purpose was to determine if MAb B72.3 demonstrated adequate sensitivity and specificity in pancreatic cancer to merit its use in a trial as an adjunct in cytologic examination and as a tool for radioimmunodetection of pancreatic cancer.

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Materials and Methods

Monoclonal Antibody

MAb B72.3 was generated by hybridoma culture obtained after the fusion of splenic lymphocytes from mice immunized with a membrane-enriched fraction of a human breast carcinoma metastasis to the liver, with a non–immunoglobulin-secreting myeloma cell line.[5,22] MAb B72.3 was obtained from Dr. David Colcher at the Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland. The purification of the MAb B72.3 has been described previously.[22]

Case Material

Paraffin-embedded blocks of formalin-fixed tissue from the Veterans Administration Medical Center, Northport, and Winthrop University Hospital, Mineola, from the period of 1973–1986 were used for this study. The material obtained for this study included the following: 25 primary adenocarcinomas of the pancreas, 16 metastatic pancreatic tumors from six patients, 8 specimens of chronic pancreatitis, and 3 specimens of adult normal pancreas. Nine of the 11 benign cases were from surgical specimens. In the autopsy material, only cases with minimal autolysis were selected. Hematoxylin and eosin sections were reviewed to confirm the histologic diagnosis.

Immunoperoxidase Assay

Fixed Tissues. From formalin-fixed paraffin-embedded tissues, 5-μm tissue sections were mounted on gelatin-coated glass slides and heated for one hour at 60 °C. A modification of the avidin–biotin–peroxidase com-

plex (Vectastain® ABC Kit, Vector Laboratories, Burlingame, CA) was used as described previously.[10] The sections were incubated overnight at 4 °C with the use of MAb B72.3 (the primary antibody) in a final concentration of 60 μg/mL. MOPC-21® (Litton Bionetics, Kensington, MD) was used as an irrelevant IgG_1 isotype negative control at an identical concentration to the primary antibody.

A semiquantitative method was used to score the slides. Only staining of strong (2+) or moderate (1+) intensity with definite brown color was considered positive. A weak stain or faint blush (±) and no staining were considered negative results. Additionally, the percentage of malignant cells reactive with MAb B72.3 was estimated by scanning the entire section and counting stained tumor cells *versus* unstained cells in representative high-power fields.

Cytologic Aspirates. Papanicolaou-stained aspirates (ten malignant and two benign) from the years 1982–1985 were used for the study. Coverslips were soaked off in xylene, and the slides were then stained in a similar fashion to fixed tissues with two exceptions: (1) the concentration of primary antibody employed was 40 μg/mL, and slides were incubated with primary antibody overnight at 4 °C; (2) slides were treated with hematoxylin for only 5 seconds to avoid overstaining of nuclei.

Results

Most of the tumors[24] were well or moderately well differentiated, only one case being poorly differentiated.

We first determined staining intensity and patterns of staining in malignant and benign pancreatic cases. Although an initial titration study indicated reactivity of malignant tumors at 20 μg/mL, we selected 60 μg/mL as the concentration because we were trying to determine maximal reactivity in the absence of background. Twenty-one of 25 malignant cases stained with strong or moderate intensity (Fig. 1). Between 10 and 100% of the cells expressed the B72.3 antigen (Fig. 2). The staining pattern was predominantly cytoplasmic, although apical and membranous staining were also seen. Heterogeneity of staining was observed in most cases (Fig. 3*A*). We had too few poorly differentiated cases to determine if there was a difference in antigen expression between well- or moderately well-differentiated and poorly differentiated carcinomas.

On the other hand, all cases of pancreatitis and normal pancreas were weakly reactive (+/−) or had negative results (Fig. 3*B*). Benign ductal cells showed faint apical staining. In two cases of chronic pancreatitis weak cytoplasmic staining of acini was seen, and in a single case weak staining of islet cells was observed.

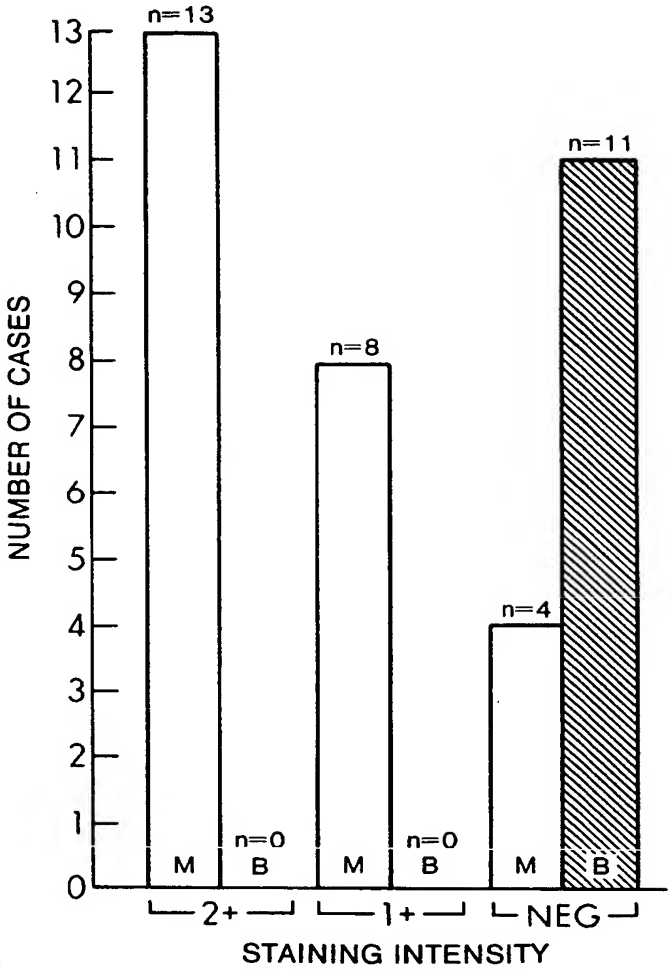


FIG. 1. Intensity of reactivity. MAb B72.3 was used at a concentration of 60 μg/mL in an overnight assay. Positive cells were graded in intensity of reactivity as follows: ++ (strong), + (moderate), +/− (weak), or 0 (negative). For purposes of scoring, +/− staining was considered negative; M = malignant; B = benign.

Table 1 compares the reactivity of primary tumors with that seen in immunoperoxidase assays of the metastatic sites. All 16 metastases of pancreatic cancer expressed the antigen. We observed that decalcification procedures do not decrease the expression of the antigen in bone marrow. Figures 4A–C demonstrated the reactivity of lymph node, bone marrow, and liver metastases. Staining patterns were similar to those observed in primary tumors.

In all cases of tumor there was a chronic inflammatory and desmoplastic reaction in surrounding benign tissue (tumor-associated pancreatitis [TAP]). Weak apical luminal staining of the duct epithelium was occasionally observed in the areas of TAP. This phenomenon may result from shedding of tumor antigen reactive with MAb B72.3.[4]

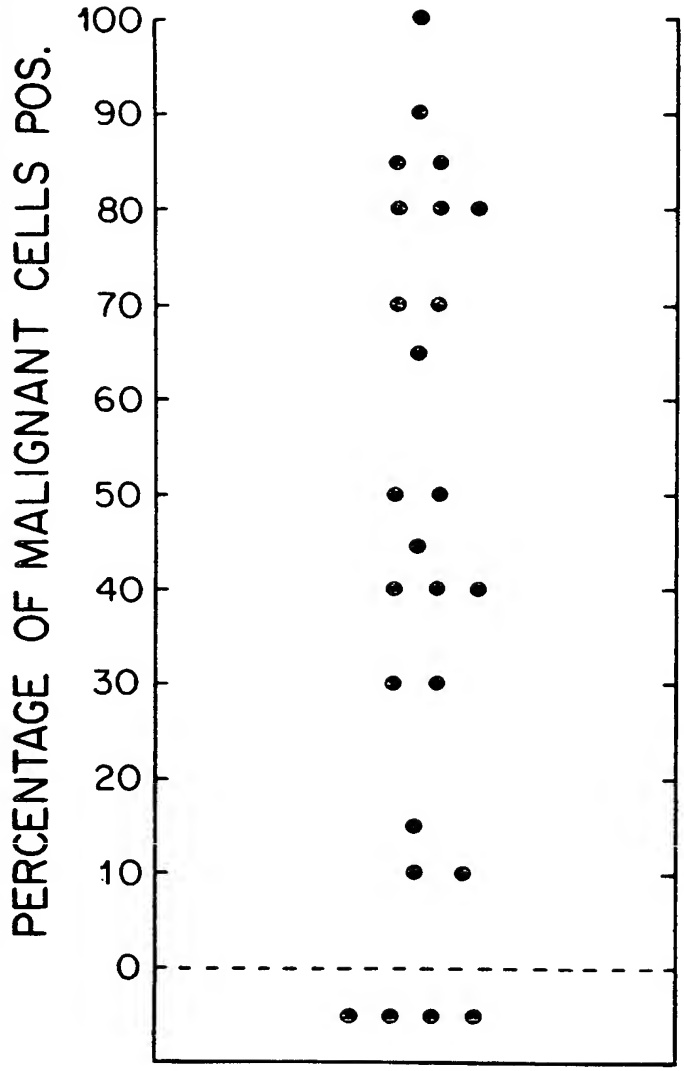


FIG. 2. Percentage tumor cells positive by immunoperoxidase assay. A semiquantitative approach was used for scoring. After scanning of the entire slide, representative high-power fields were used to further estimate stained *versus* unstained tumor cells.

A Papanicolaou smear obtained from a computed tomography (CT)–directed fine-needle aspiration biopsy of a pancreatic adenocarcinoma was superstained with MAb B72.3. Reactivity was noted in malignant duct cells (Fig. 5). In view of this finding, an additional nine malignant and two benign cytologic cases were stained in similar fashion. All malignant cases reacted with moderate to strong intensity, with a range of 25–75% of tumor cells staining positively. One case originally diagnosed as atypia by cytologic examination stained strongly with the MAb. The biopsy confirmed the presence of a well-differentiated adenocarcinoma.

Discussion

The selective expression of the cell surface TAG-72 antigen in primary and metastatic pancreatic cancer, in contrast to weak or no expression in pancreatitis or benign pancreas suggests that MAb B72.3 may be of value as a diagnostic adjunct in cytologic aspirates of pancreatic cancer as well as radioimmunodetection.

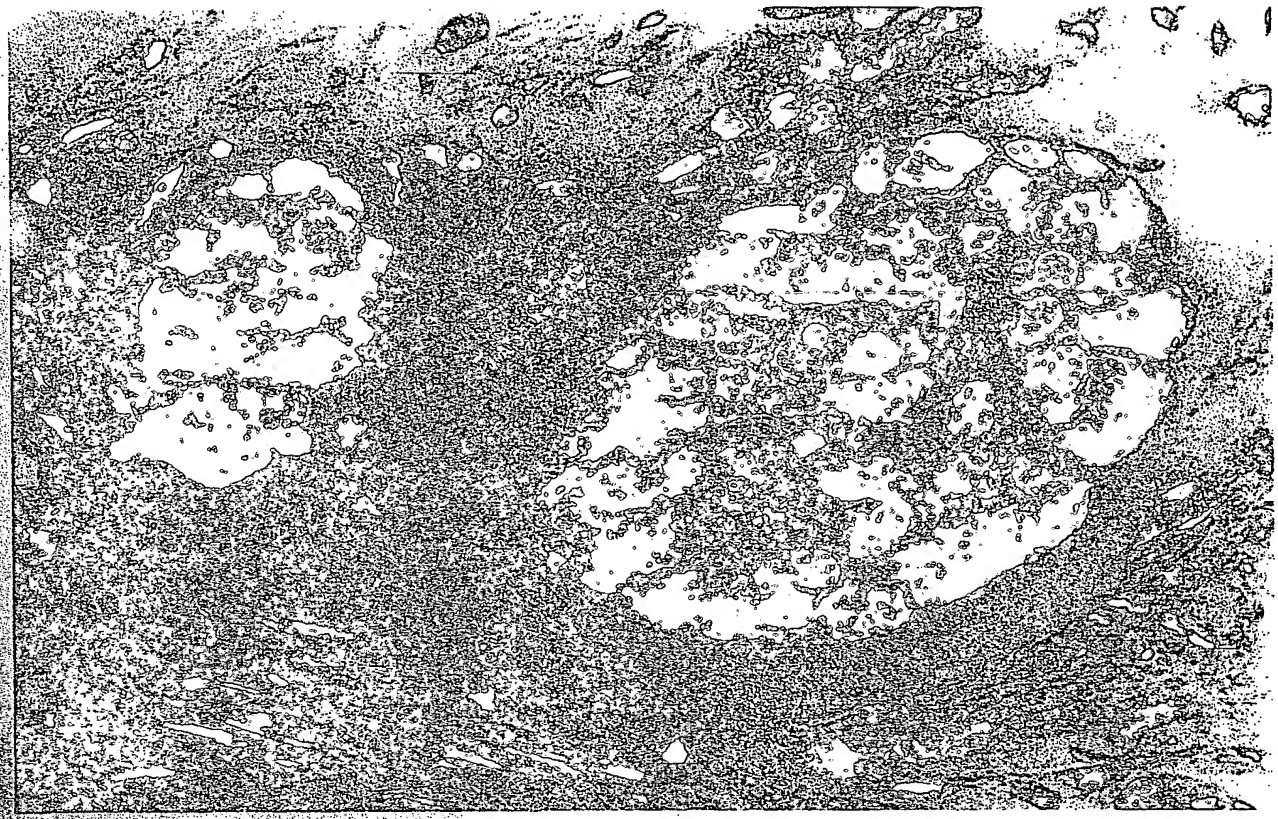
Other MAbs reactive to pancreatic cancer have been generated with the use of human pancreatic cancer cell lines as the immunogen. The DU-PAN antibodies react with either normal pancreas or fetal pancreas. DU-PAN-2, an IgM, reacts with carcinomas outside of the gastrointestinal tract but to a lesser degree than MAb B72.3. DU-PAN-1 and DU-PAN-3 are more pancreas specific.[1,12,16] MAb AR 1-28 is reactive to a membrane-associated antigen of molecular weight of 200,000 daltons. It reacted to 23 of 27 pancreatic cancers tested, demonstrating a predominant apical staining pattern in well-differentiated and moderately well-differentiated tumors. It was less reactive to poorly differentiated cancers.[3]

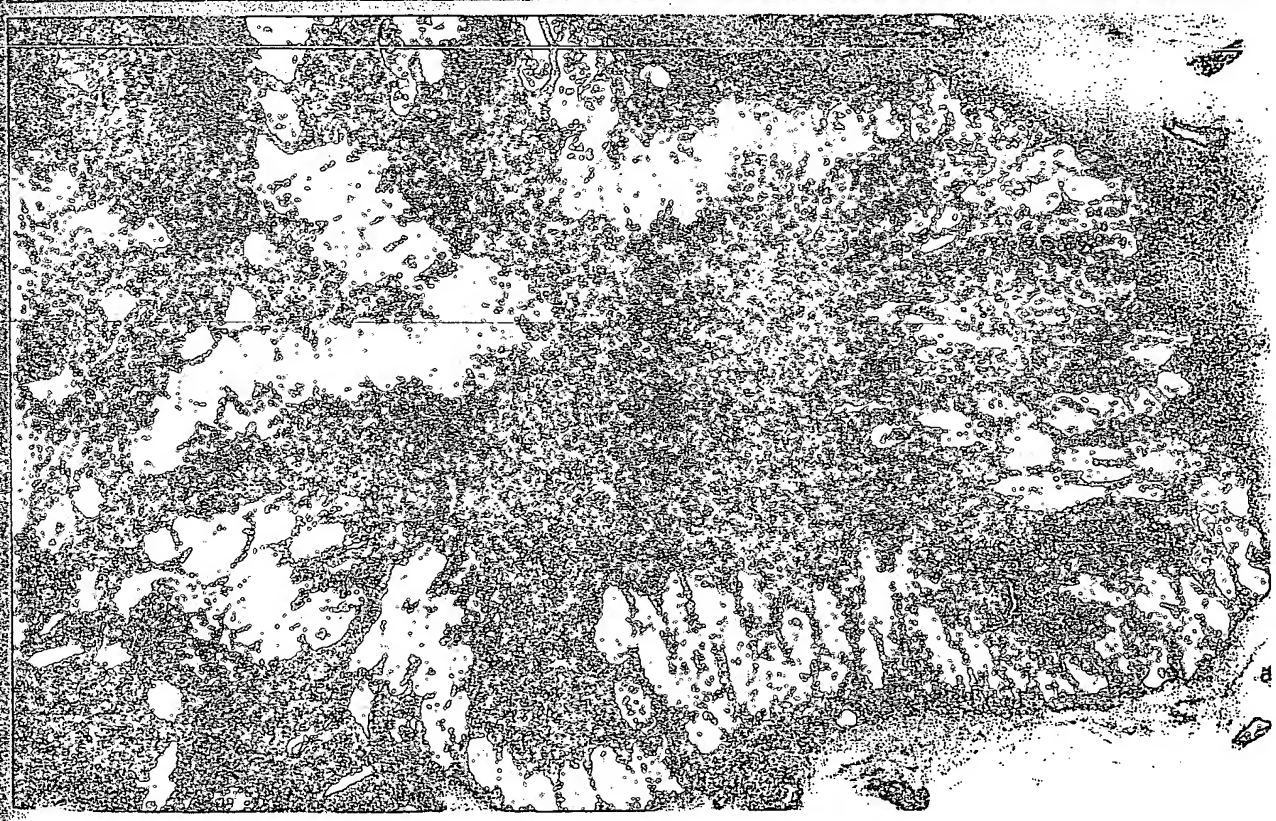
MAB AR 1-28 did not react with normal pancreatic, ductal, and islet cells.[2] Its reactivity to metastatic pancreatic cancer and application to clinical aspiration cytologic examination were not studied.

The early diagnosis of pancreatic carcinoma can be made by aspiration cytologic examination with diagnostic accuracy between 60 and 90% among experienced cytopathologists.[7–9,15,19,20] The major problems encountered are as follows: (1) very well differentiated adenocarcinomas are difficult to distinguish from atypical and reactive ductal cells as seen in severe pancreatitis; (2) some pancreatic carcinomas have a marked scirrhous component; the malignant cells are not released from their fibrous matrix, with resultant sparse cells on the slide.

Although we tested it on only 12 pancreatic aspirates, we were encouraged by seeing no staining of benign ductal cells, similar to what is observed on fixed tissue sections. In addition, in 1 of 10 malignant cases it proved to be a useful diagnostic adjunct to the cytopathologist. Other authors have suggested its diagnostic value in adenocarcinoma in pleural effusions[23] and breast aspirates.[14]

FIG. 3. *A* (*upper*). Primary pancreatic cancer. Positive cytoplasmic staining of adenocarcinoma of pancreas (×400). Note heterogeneity of staining. *B* (*lower*). Benign pancreas. Ductal cells from benign pancreas demonstrating absence of reactivity to MAb B72.3 (×400).





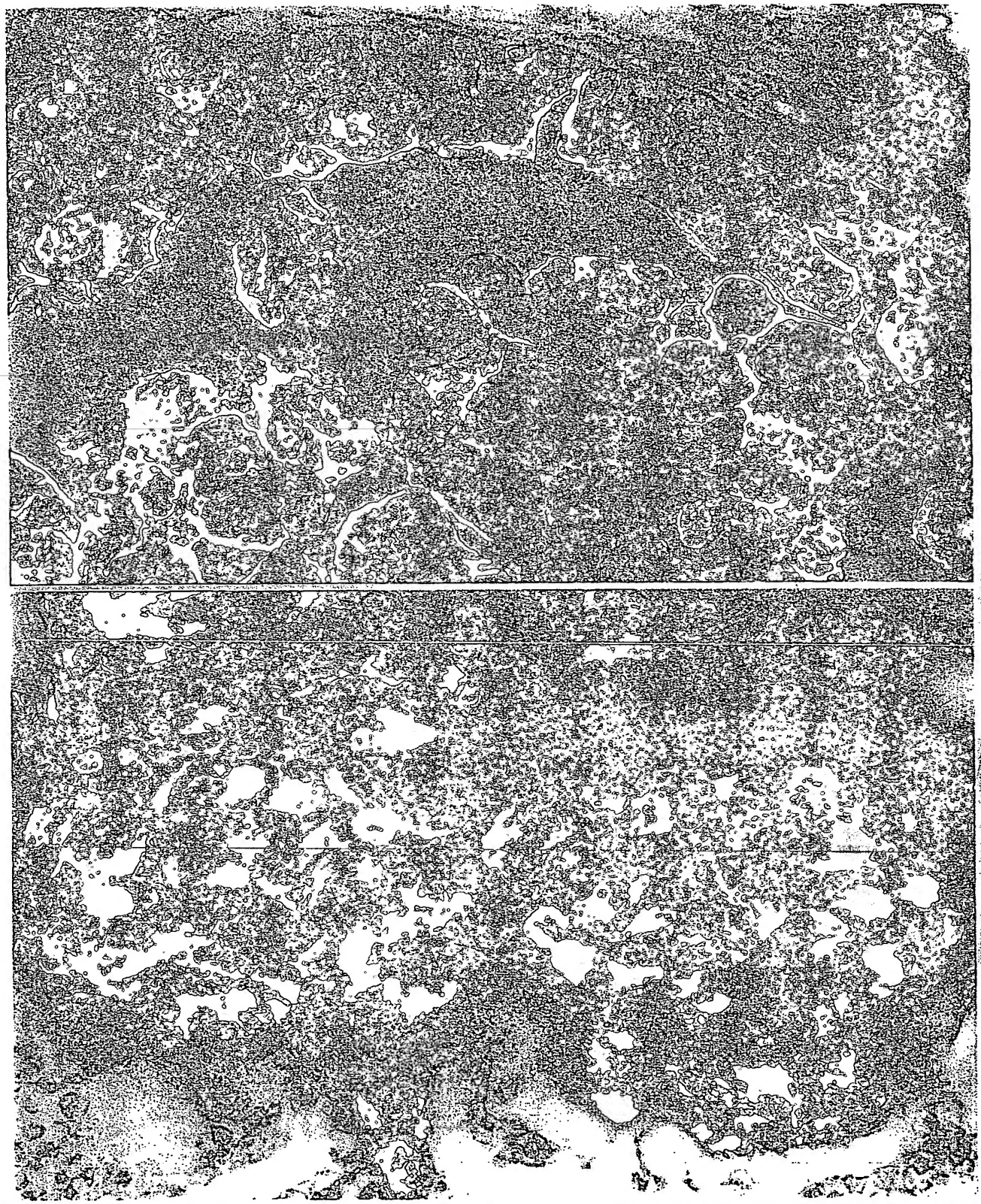


FIG. 4. *A* (*upper*). Regional node metastasis. Strong apical and membranous staining of a regional lymph node metastasis (×400). *B* (*lower*). Bone marrow metastases. Cytoplasmic staining of bone marrow metastasis of pancreatic adenocarcinoma (×400). (*continued.*)

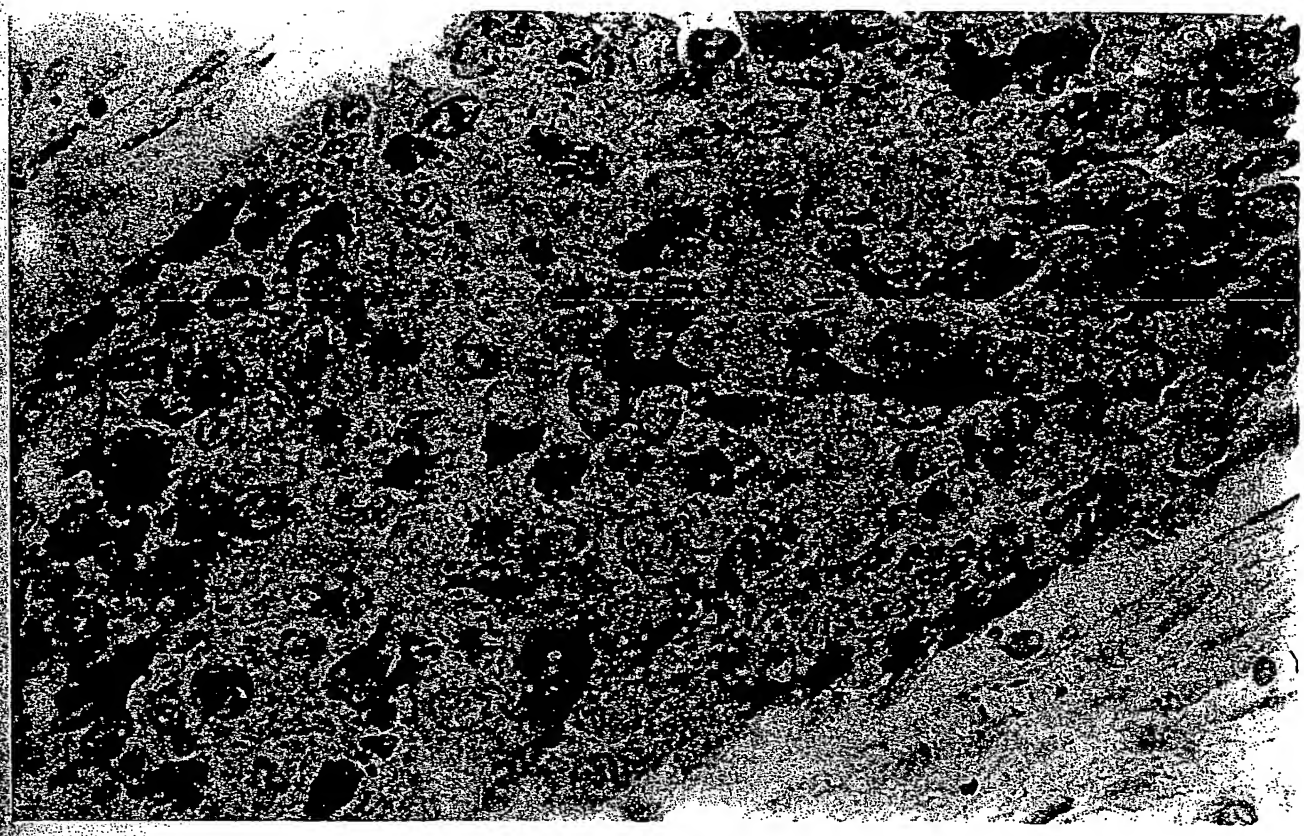


FIG. 4 (*continued*). *C.* Liver metastasis. Heterogeneous cytoplasmic staining of tumor embolus in portal venule (×400).

Assessment of extent of disease in pancreatic cancer requires several diagnostic modalities (ultrasound, CT scan, endoscopic retrograde cholangiopancreatography),[6,17] but at present the extent of regional disease and even liver metastases is often underestimated before operation. The encouraging aspect of our study is that all regional and distant metastases express the TAG-72 antigen to at least an equivalent degree as expressed in the primary tumor. In addition, if we include the aspirated material, 28 of 35 (80%) primary cancers had at least 25% of tumor cells staining positively. Initial radioimmunodetection studies in patients with colon

Table 1. Reactivity of Primary Tumors and Metastatic Sites

Primary*	Metastases†							
1. 80 / +	Lymph node 90 / +	Bone 80 / ++	Lung 95 / ++	Liver 85 / +	Adrenal 65 / +	Small intestine 95 / ++	Colon 100 / ++	Brain 65 / ++
2. 10 / ++				Liver 5 / +				
3. 40 / ++		Bone 50 / ++	Lung 80 / +	Liver 40 / ++				
4. 85 / ++		Bone 90 / ++			Adrenal 90 / ++			
5. 100 / ++	Lymph node 100 / ++							
6. 85 / ++		Bone 100 / ++						

* Primary adenocarcinomas (1–6). Top figure: Percentage of cells staining positively. Bottom figure: Intensity of staining. Scoring method has been described in text.

† Metastatic sites. Represented in an identical fashion to the primary sites.

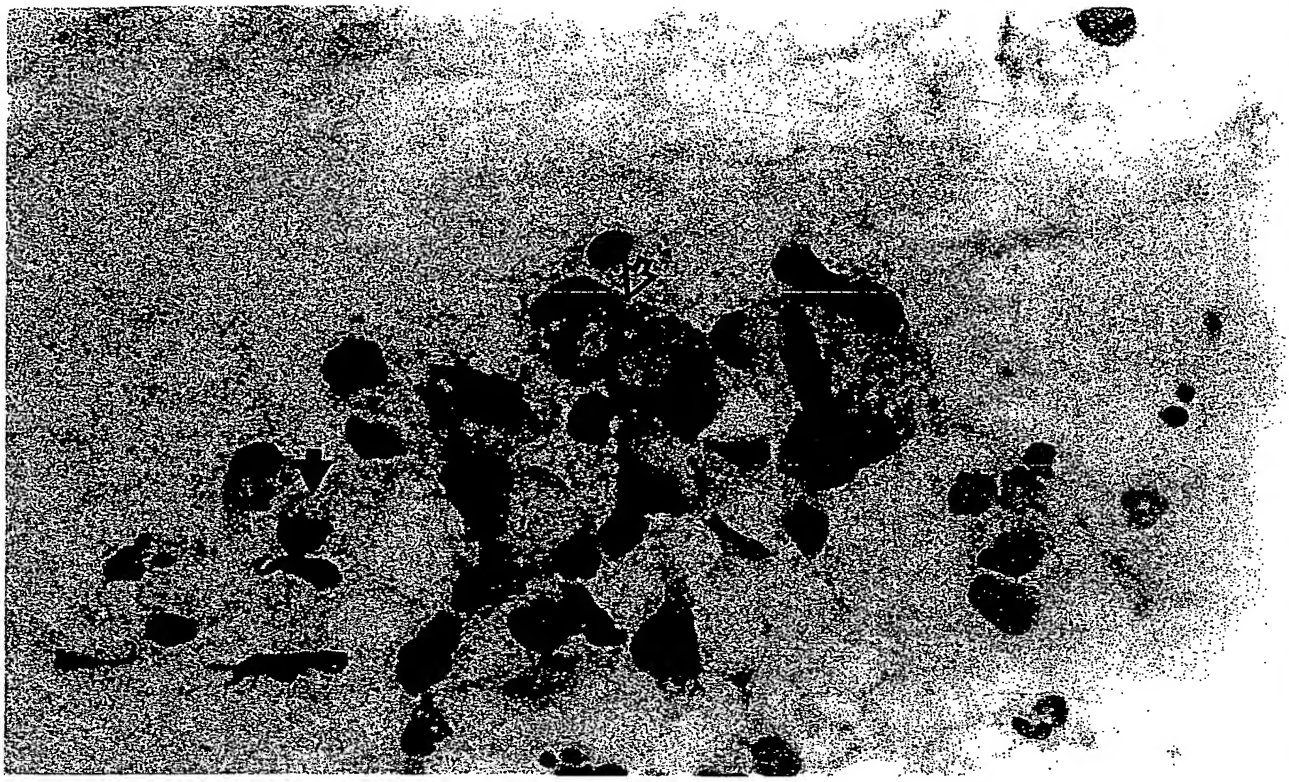


FIG. 5. Aspirate of pancreatic mass. *Open arrow* indicates positive cytoplasmic staining of malignant cells (×400). *Closed arrow* constrasts an unstained benign cell.

cancer with the use of ^{131}I B72.3 to detect metastases have demonstrated good tumor-to-blood and tumor-to-normal tissue ratios, with imaging possible in more than 50% of patients.[4] Larson and associates have demonstrated the ability to image melanoma with radiolabeled antitumor antibodies.[13] Such studies should serve as an impetus for clinical trials using radiolabeled B72.3 $F(ab')_2$ to detect spread of pancreatic cancer before operation.

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